Original Paper

Cypermethrin-induced Histochemical Changes in Liver of Albino Rats

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ABSTRACT

Cypermethrin is a synthetic pyrethroid insecticide that has high insecticidal activity, low avian and mammalian toxicity, and adequate stability in air and light.

This paper is an attempt to study its toxicity *in vivo* in rat liver at cellular level.

Following exposure to oral, sublethal doses (41 mg/kg bw) of cypermethrin as single dose, double dose and multiple dose with 48 h interval, the histochemical changes were studied in different groups of rat livers. Histochemical examination indicated depletion of polysaccharides in the liver. This change occurred in a dose and time-dependent manner in treated rat liver.

Key Words: Cypermethrin; Histochemistry; Rat liver

Introduction

Pesticides are widely used throughout the world in agriculture to protect food crops, and in public health to control diseases transmitted by vectors or intermediate hosts. An increase in global food demand has resulted in a significant increase in the use of pesticides in agriculture. This has caused great concern among health and environmental scientists, since some of these chemicals induce mutations (somatic as well as germ-line) in experimental systems.[1] In humans, exposure to pesticides has been associated with cancer.[2]

Synthetic pyrethroid pesticides account for over 30% of the global pesticide use.[3] Two distinct classes of pyrethroids have been identified based on different behavioural, neuropsychological and biochemical profiles. Type I pyrethroids mainly cause hyper-excitation and fine tremors, while Type II pyrethroids possess a cyano-group and produce a more complex syndrome, including seizures.[4] These compounds have gained popularity over organochlorine and organophosphate pesticides due to their high efficacy against target species,[5] their relatively low mammalian toxicity,[6] and rapid biodegradability.[7]

Cypermethrin [alpha-cyano-3-phenoxybenzyl ester of 2, 2-dimethyl-3-(2, 2-dichlorovinyl) cyclopropane carboxylic acid], is a composite synthetic pyrethroid. It is a broad spectrum, biodegradable insecticide, and a fast-acting neurotoxin with good contact and stomach action. It is used to control many pests, including moths, and pests of cotton, fruit and vegetable crops. Consistent with its lipophilic nature, cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain.[8]

Cypermethrin has been classified by the US Environmental Protection Agency as a possible carcinogen.[9] The pesticide has been shown to induce chromosomal aberrations and micronucleus formation in mouse bone marrow, as well as in spleen.[10,11] It also increases the frequency of sister chromatid exchange in bone marrow cells of mice.[12] DNA damage has been observed in lymphocytes of workers occupationally exposed to pesticides such as cypermethrin.[13]

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The present study critically examines the histochemical changes in rat liver treated with sub-lethal doses of cypermethrin, in an attempt to extrapolate the effects to farmers, pesticide applicators, industrial workers and other pesticide users who are exposed to the pesticide repeatedly.

Materials and Methods

Test Chemical: Technical grade cypermethrin (92% purity; *cis:trans* ratio 40:60) was obtained from Tagros Chemicals India Limited, Chennai.

Experimental Animals: About 20 adult, healthy, wistar strain albino rats (70±5 days, 175±10 g) were obtained from the Indian Institute of Science (Bangalore, India) breeding colony, and raised on a commercial pellet diet (Sai Durga Feeds and Foods, Bangalore, India), and water *ad libitum.* The animals were housed at constant temperature (28±2ºC) and relative humidity (60±10%), with a 12 h light–12 h dark cycle.

Experimental Design: The study design comprised four groups consisting of five rats each. Toxicity evaluation was conducted by static bioassay method,[14] and the LD_{50} for 48 h value of cypermethrin to rats was found to be 205 mg/kg bw. 1/5 LD_{50} value (41 mg/kg bw) was selected as sub-lethal dose and administered as single, double and multiple doses with one day interval in between. The first group of animals was treated as vehicle controls, and administered corn oil. To the second group of animals, single dose of cypermethrin (i.e., on 1st day) was administered orally (41 mg/kg bw). Double doses (82 mg/kg bw) were given with 48 h interval to the third group of animals on 1st and 3rd day. To the fourth group of animals, multiple doses (164 mg/kg bw) were given with 48 h interval, i.e., on 1st, 3rd, 5th and 7th day. After 48 h, both control and experimental animals were sacrificed and liver tissues isolated and fixed in 10% buffered formalin for further processing.

Histochemical Study: Liver was removed and small pieces were fixed in 10% buffered formalin. The fixed samples were dehydrated in ascending series of ethanol, cleared in methyl benzoate, and embedded in paraffin wax. Sections of 6 µ thickness were cut, mounted and stained with periodic acid Schiff's (PAS) technique for demonstration of polysaccharides (liver glycogen).[15]

Results

Examination of liver sections of control rats stained with periodic acid Schiff's (PAS) showed the distribution of polysaccharide inclusions in the form of purple granules and particles in the cytoplasm of the hepatocytes (**Fig A**).

In single dose cypermethrin administration, mild depletion of polysaccharide content of hepatocytes was observed (**Fig B**). This diminution was quite evident in the amount and stainability.

In double dose administration, moderate depletion in the polysaccharide content of the hepatocytes was observed (**Fig C**).

Under multiple dose administration, severe depletion in the polysaccharide content of the hepatocytes was observed (**Fig D**). In these specimens, polysaccharide content displayed faint stainability and were hardly detectable. This change was in the form of a dose and time-dependent manner in treated rat liver.

Discussion

Pesticides are used extensively in agriculture and their residues have affected the environment adversely. The use of such biologically active compounds poses potential problems of toxicity among those who manufacture, formulate, or use these compounds. Pesticides are also used directly in aquaculture to control ectoparasites and insects in nurseries and grow-out systems.

The liver is the centre for detoxifying any foreign compounds entering the body. So, it is invariably exposed to a wide variety of exogenous and endogenous products. These include environmental toxins and chemicals present in food or drink.[16]

Carbohydrates and lipids function primarily as sources of energy (metabolic fuels), but also have a non-fuel function in the body.[17] Catabolism of polysaccharides makes major contribution to the total energy production in rats. The depletion of polysaccharide content observed in this investigation (**Fig B**, **C** and **D**) attest to this fact.

These results are in agreement with earlier reports of Farrag and Shalby,[18] and Sakr and Jamal Al Lail,[19] who demonstrated a similar situation in albino rat, and *Clarias gariepinus* exposed to different pesticides. Reddy et al[20] reported that fenvalerate altered glycogen metabolism in liver and muscles of *Cyprinus carpio*. Singh and Srivastava[21] demonstrated that carbohydrates decreased as a result of exposure to sub-lethal concentration of a mixture of aldrin and formothion. These insecticides in-

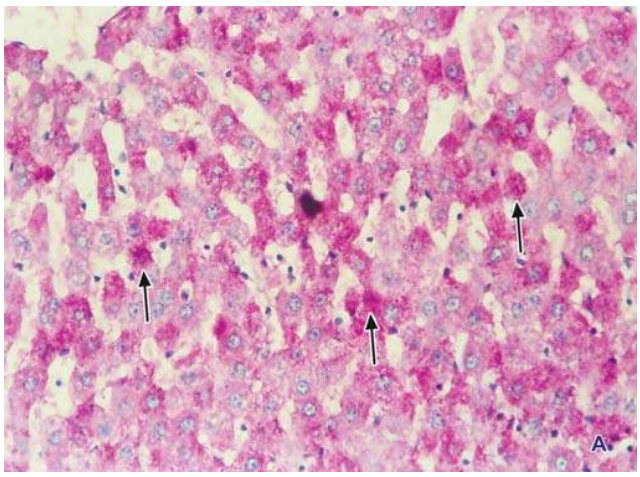

Fig A: Control rat liver showing polysaccharide particles accumulated in the cytoplasm of hepatocytes. (PAS x 200)

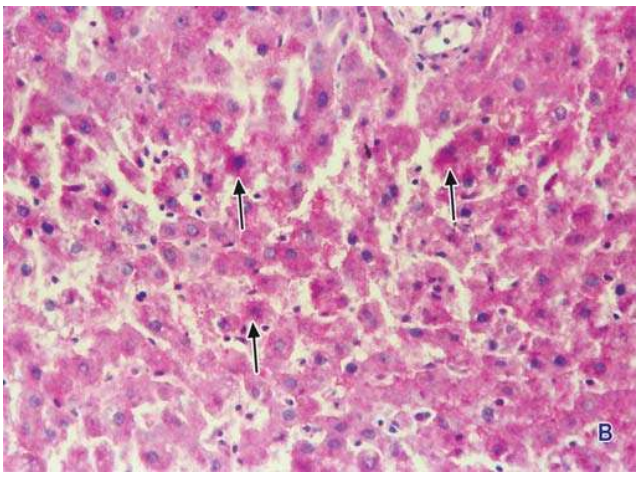

Fig B: Single dose cypermethrin-administered rat liver showing mild depletion in polysaccharide content. (PAS x 200)

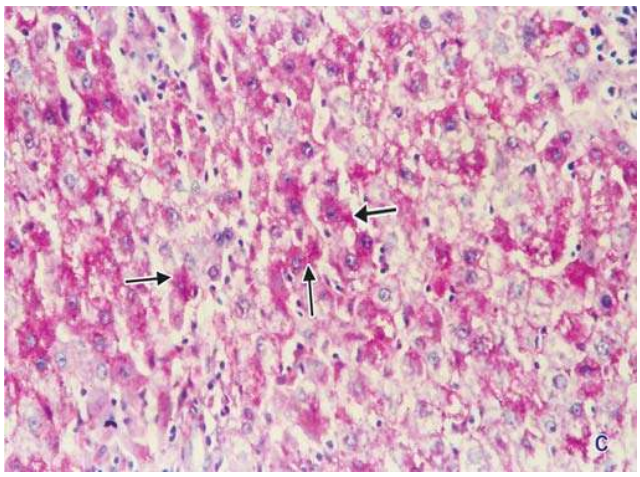

Fig C: Double dose cypermethrin-administered rat liver showing moderate depletion in polysaccharide content. (PAS x 200)

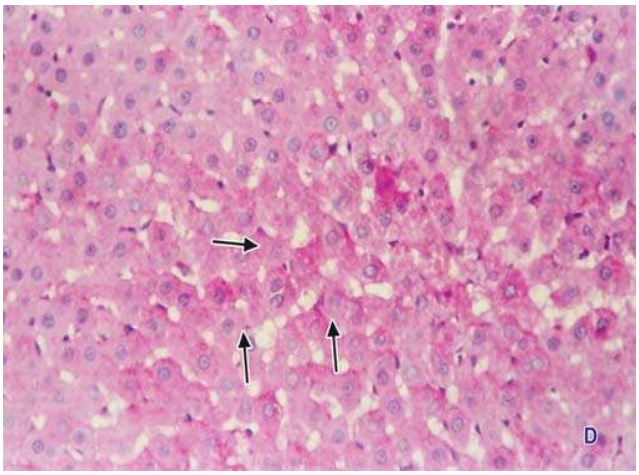

Fig D: Multiple dose cypermethrin-administered rat liver showing severe depletion in polysaccharide content. (PAS x 200)

duced marked diminution in the glycogen content of the liver and muscles.

Exposure of freshwater fish *Mystus vittatus* to sub-lethal concentrations of the two pesticides thiotox and dichloruos for one month was found to induce marked depletion in both liver and muscle glycogen.[22] Sub-lethal concentrations of quinalphos resulted in reduction of glycogen in the liver of *Channa punctatus*.[23] The activity levels of succinate dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G6PD) were assessed in various tissues of *Cyprinus carpio* exposed to lethal concentrations of different pyrethroids, including fenvalerate for a period of 72 h. The results indicated a steady decrease in SDH activity with a concomitant increase in G6PD activity. The decreased SDH activity indicated inhibition of SDH at mitochondrial level, and the increased G6PD activity an enhancement of an alternative pathway of carbohydrate metabolism, viz the hexose monophosphate shunt (HMP) or pentose phosphate pathway as a biochemical adaptation to overcome the toxic stress.[24] Glycogen depletion in liver and muscle after toxic stress has been reported in several studies with aquatic animals.[25,26]

In general, the reduced carbohydrate components due to the effect of pesticides could be due to the release of hydrolytic enzymes from ruptured lysosomes because of the toxic effect of deleterious agents.[27,28]

The present study proves that cypermethrin affects the histochemical content of the liver of albino rats. It is evident that long term exposure to sub-lethal doses of pyrethroid pesticides can result in cell metabolism poisoning.

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